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Corynebacterium lipophiloflavum sp. nov. isolated from a patient with bacterial vaginosis

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Abstract

A unique coryneform bacterium was isolated from a patient with bacterial vaginosis. Chemotaxonomical investigations demonstrated that the unknown bacterium belonged to the genus *Corynebacterium*. The yellow-pigmented, slightly lipophilic, oxidative, urea-hydrolyzing bacterium could be phenotypically readily differentiated from the other members of the genus *Corynebacterium*. Comparative 16S rRNA gene analysis revealed that the bacterium represented a new subline within the genus *Corynebacterium* for which the name *Corynebacterium lipophiloflavum* sp. nov. is proposed. The type strain is CCUG 37336 (DSM 44291).

Keywords: *Corynebacterium lipophiloflavum* sp. nov.; *Corynebacterium* taxonomy; Bacterial vaginosis

1. Introduction

Bacterial vaginosis (BV) is a polymicrobial process involving *Gardnerella vaginalis* and anaerobic bacteria such as *Prevotella* spp., *Mobiluncus* spp., and *Peptostreptococcus* spp. as possible etiologic agents [1]. During the search for further bacterial pathogens involved in BV we isolated a unique coryneform bacterium which was further characterized. Investigations applying both phenotypic and molecular genetic methods revealed that the isolate represented a new *Corynebacterium* species.

Within the genera of coryneform bacteria the genus *Corynebacterium* comprises at present the largest number of single species, with eleven species having been defined within the last ten years [2,3]. This report adds further evidence to the enormous diversity within the genus *Corynebacterium*.

2. Materials and methods

2.1. Microorganism and cultivation

Strain DMMZ 1944, now named *C. lipophiloflavum*, was initially isolated from a vaginal swab which, apart from the other media used, was plated

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Table 1
Characteristics differentiating *C. lipophiloflavum* from other lipophilic *Corynebacterium* spp.[a]

Species	Type of metabolism[b]	Nitrate reduction	Urea hydrolysis	Pyrazinamidase	Alkaline phosphatase	Acid production[c]				TBSA[d]	Pigment
						Glu	Mal	Suc	Man		
C. lipophiloflavum	O	−	+	+	+	−	−	−	−	−	Yellow
C. accolens	F	+	−	V[e]	−	+	−	V	V	−	−
C. afermentans subsp. *lipophilum*	O	−	−	+	+	−	−	−	−	−	−
C. bovis	O	−	−	V	+	+	−	−	−	+	−
C. diphtheriae biotype *intermedius*	F	+	−	−	−	+	+	−	−	−	−
C. jeikeium	O	−	−	+	+	+	V	−	−	−	−
C. macginleyi	F	+	−	−	+	+	−	+	V	−	−
C. urealyticum	O	−	+	+	V	−	−	−	−	+	−
CDC group F-1	F	V	+	+	−	+	+	+	−	−	−
CDC group G	F	V	−	+	+	+	V	V	−	V	−

[a]Data from reference [2].
[b]O, oxidative; F, fermentative.
[c]Glu, glucose; Mal, maltose; Suc, sucrose; Man, mannitol.
[d]TBSA, tuberculostearic acid.
[e]V, variable.

on sheep blood agar (SBA) and incubated for 24 h in a 5% CO_2-enriched atmosphere. The vaginal swab had been taken from a 32-year-old female with the clinical diagnosis of BV. *G. vaginalis* and *Peptostreptococcus* spp. were cultured from the same specimen in addition to strain DMMZ 1944.

2.2. Phenotypical analysis

The biochemical profile (enzymatic reactions, acid production from carbohydrates) of strain DMMZ 1944 was determined as described previously [4]. Chemotaxonomic investigations included determination of the cellular fatty acids (CFA), the diamino acid of the peptidoglycan, the presence of mycolic acids, and the G+C content [3]. The antimicrobial susceptibility pattern was determined by using the MCN system (Merlin Diagnostics, Bornheim, Germany) as outlined before [5].

2.3. Phylogenetic analysis

Determination of the 16S rRNA gene sequence of strain DMMZ 1944 (EMBL accession number Y09045) was by PCR-mediated amplification of the gene followed by cycle sequencing of the PCR product, and reading the reaction products on a model 373A automatic sequencer (Applied Biosystems, Foster City, USA) as described previously [6]. Phylogenetic analysis included alignment, calculation of percentage sequence similarity, construction of a phylogenetic tree, and an assessment of tree topology by bootstrap analysis as has also been outlined previously [6].

3. Results and discussion

Strain DMMZ 1944 formed tiny, non-hemolytic colonies of less than 0.2 mm in diameter after 24 h incubation on SBA at 37°C. Colonies were larger (up to 1 mm in diameter) when strain DMMZ 1944 was grown on SBA supplemented with 1% Tween 80, i.e., the strain was lipophilic [2]. The colonies showed an intense yellow pigment. The Gram stain showed typical club-shaped gram-positive rods of 1–3 μm in length which were arranged as single cells, as pairs, or in clusters. Strain DMMZ 1944 was not partially acid-fast.

The basic phenotypical screening reactions [7] of strain DMMZ 1944 were as follows: catalase positive (which separated the strain from the catalase-negative *G. vaginalis*); non-motile; oxidative metabolism; nitrate reduction negative; urea hydrolysis

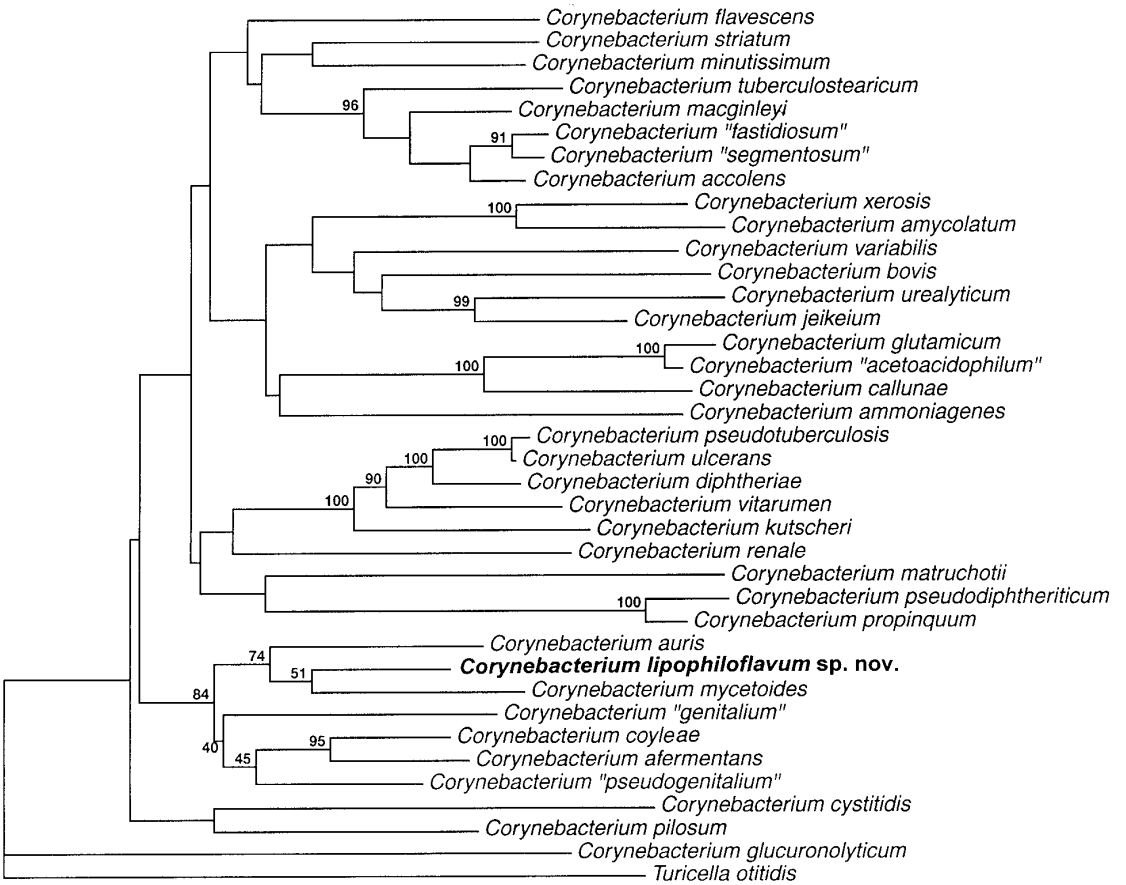

Fig. 1. Unrooted tree showing the phylogenetic position of *C. lipophiloflavum* within the genus *Corynebacterium* sensu stricto. The tree constructed using the neighbor-joining method was based on a comparison of approximately 1330 nucleotides. Bootstrap values, expressed as a percentage of 250 replications, are given at the branching points.

positive; esculin hydrolysis negative; acid was not produced from glucose, maltose, sucrose, mannitol, and xylose; CAMP reaction negative. With these few characteristics only, strain DMMZ 1944 could be readily differentiated from all other presently defined lipophilic *Corynebacterium* spp., in particular *C. urealyticum* (see Table 1).

Analysis of CFAs revealed C16:0 (28% of total CFAs), C18:1ω9*c* (38%), and C18:0 (23%) as the major components but tuberculostearic acid (TBSA), which is always present in *C. urealyticum* strains [8,9], was not detected. *Meso*-diaminopimelic acid was detected as the diamino acid of the peptidoglycan. Short-chain mycolic acids were present. The G+C content of the bacterial DNA was 65 mol%. Based on these findings strain DMMZ 1944 could be assigned to the genus *Corynebacterium* only [10].

The commercial API Coryne identification system gave the numerical code 2101004 which corresponded with the identification of the isolates as *C. urealyticum* (previously CDC group D-2 bacteria [11]), an urogenital pathogen [12], with an 98.2% identification score (an estimate of how closely the profile corresponds to the taxon relative to all other taxa in the data base) and a T index of 0.95 (an estimate of how closely the profile corresponds to the most typical set of reactions for each taxon). However, the characteristics differentiating strain DMMZ 1944 from the non-pigmented *C. urealyticum* have been outlined above. In addition, it was noted that the unknown *Corynebacterium* strain hy-

Table 2
Levels of 16S rRNA sequence similarity between *C. lipophiloflavum* sp. nov. and other *Corynebacterium* species as well as *Turicella otitidis*

Species	% 16S rRNA sequence similarity with *C. lipophiloflavum* sp. nov.	Species	% 16S rRNA sequence similarity with *C. lipophiloflavum* sp. nov.
C. accolens (X80500)[a]	94.6	*C. matruchotii* (X84443)	92.2
C. 'acetoacidophilum' (X84240)	93.0	*C. minutissimum* (X84678)	94.5
C. afermentans (X81874)	96.6	*C. mycetoides* (X84241)	97.2
C. ammoniagenes (X84440)	94.4	*C. pilosum* (X84246)	95.8
C. amycolatum (X84244)	94.2	*C. propinquum* (X84438)	93.7
C. auris (X82493)	97.1	*C. pseudodiphtheriticum* (X84258)	93.6
C. bovis (X84444)	93.7	*C. 'pseudogenitalium'* (X81874)	96.2
C. callunae (X84251)	93.4	*C. pseudotuberculosis* (X84255)	94.1
C. coyleae (X96497)	96.7	*C. renale* (X84249)	94.6
C. cystitidis (X84252)	94.7	*C. 'segmentosum'* (X84437)	95.2
C. diphtheriae (X84248)	94.9	*C. striatum* (X84442)	94.8
C. 'fastidiosum' (X84245)	94.9	*C. 'tuberculostearicum'* (X84247)	95.2
C. flavescens (X84441)	95.2	*C. ulcerans* (X84256)	94.3
C. 'genitalium' (X84253)	95.7	*C. urealyticum* (X84439)	93.5
C. glucuronolyticum (X86688)	93.3	*C. variabilis* (X53185)	93.4
C. glutamicum (X84257)	93.4	*C. vitarumen* (X84680)	93.8
C. jeikeium (X84250)	94.0	*C. xerosis* (X84446)	93.9
C. kutscheri (X81871)	94.0	*T. otitidis* (X73976)	91.5
C. macginleyi (X80499)	94.8		

[a]The numbers in parentheses are EMBL 16S rRNA nucleotide sequence accession numbers.

drolyzed urea more slowly (after 12 h in Christensen's urea broth [13]) than *C. urealyticum* strains (usually positive after only 5 min in Christensen's urea broth). Further enzymatic activities of strain DMMZ 1944 included esterase, esterase lipase, lipase, leucine arylamidase, acid phosphatase, and phosphoamidase whereas valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *n*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase could not be detected. In our experience, the presence of lipase activity is very rarely observed in other true *Corynebacterium* spp. (G. Funke, unpublished data).

Antimicrobial susceptibility testing demonstrated that the strains were susceptible to (minimal inhibitory concentrations given in μg/ml) amikacin (1.0), amoxicillin (0.25), amoxicillin/clavulanic acid (≤0.0625), azithromycin (0.0625), cefazolin (<0.125), cetriaxone (0.5), cefuroxime (0.5), chloramphenicol (2.0), ciprofloxacin (0.25), clarithromycin (<0.03), clindamycin (0.125), erythromycin (≤0.03), fusidic acid (0.125), gentamicin (0.125), imipenem (0.0625), meropenem (0.25), netilmicin (0.125), norfloxacin (2.0), oxacillin (1.0), penicillin (0.125), piperacillin (4.0), rifampin (0.0625), teicoplanin (0.5), tetracycline (2.0), tobramycin (0.125), sparfloxacin (0.25), and vancomycin (0.5) but resistant to aztreonam (>64), cefetamet (64), and fosfomycin (>256) [14]. In contrast, *C. urealyticum* is almost ever multiresistant including resistance to β-lactams, penems, aminoglycosides, macrolides, clindamycin, rifampin, and quinolones [15]. Due to the fact that the unknown *Corynebacterium* exhibited very low MICs for nearly all clinically applied antimicrobial agents it is not unlikely that it, like other fastidious lipophilic corynebacteria, might not be recovered and, therefore, underestimated in clinical specimens after administration of antibiotics. However, the etiologic role of the unknown *Corynebacterium* in our patient with BV remained unclear.

In order establish the precise phylogenetic position and the genealogical distinctiveness of the unknown *Corynebacterium* the gene encoding the 16S rRNA was amplified by PCR and subjected to sequence analysis. The almost complete 16S rRNA gene sequence (ca. 1400 bp) was determined. The unknown bacterium clustered within the genus *Corynebacte-*

rium sensu stricto, where it formed a distinct subline (Fig. 1). The non-lipophilic, oxidative *C. auris* and the non-lipophilic, fermentative *C. mycetoides* were the closest phylogenetic neighbors of the unknown *Corynebacterium* showing ca. 3% 16S rRNA sequence divergence (Table 2). Based on the phenotypic and molecular genetic distinctiveness of the unknown bacterium we formally propose a new *Corynebacterium* species, *Corynebacterium lipophiloflavum* sp. nov., for the strain studied.

3.1. Description of *Corynebacterium lipophiloflavum* sp. nov.

Corynebacterium lipophiloflavum (li'po.phi.lo.fla.vum. Gr. n. *lipos*, fat; Gr. adj. *philos*, loving; L. adj. *flavus*, yellow; M.L. adj. *lipophiloflavum*, fat loving and yellow) cells are gram-positive, asporogenous, club-shaped, 1 to 3 μm in length, and nonmotile. Colonies are yellowish, circular, convex, slightly dry, and less than 0.2 mm in diameter after 24 h of incubation on SBA but colonies are up to 1 mm in diameter when cells are grown on SBA supplemented with 1% Tween 80. Catalase positive. Acid is not produced from glucose, maltose, sucrose, mannitol, xylose, lactose, and glycogen. Nitrate is not reduced. Urea is slowly hydrolyzed but esculin is not hydrolyzed. The CAMP reaction is negative. Pyrazinamidase, alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, acid phosphatase, and phosphoamidase activities are detected but pyrrolidonyl arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *n*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are not detected.

The cell wall contains *meso*-diaminopimelic acid. Mycolic acids are present. The main straight-chain saturated fatty acids are palmitic and stearic acids; oleic acid is the predominant unsaturated fatty acid. The DNA base composition is 65 mol% G+C. Isolated from a patient with bacterial vaginosis. The type strain has been deposited in the Culture Collection of the University of Göteborg, Sweden, as CCUG 37336, and in the German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, as DSM 44291.

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